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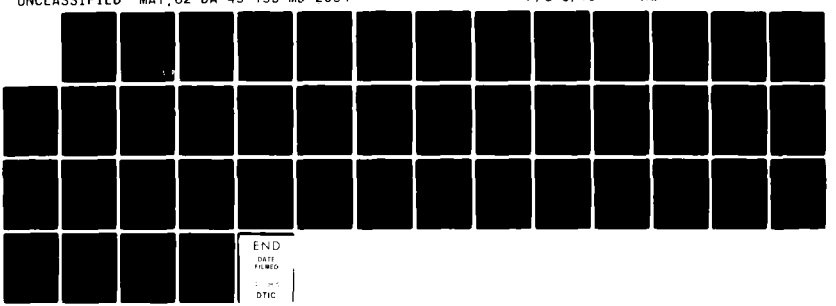

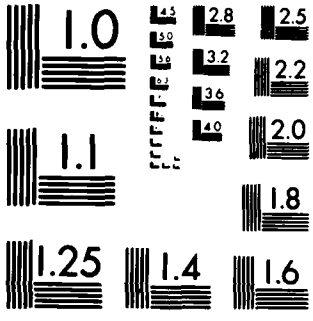

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BIOCHEMICAL CHANGES IN TISSUES DURING INFECTIOUS ILLNESS: BIOENERGETICS OF INFECTION AND EXERCISE

Annual Progress Report No. 16
(For the period 1 July 1980 to 31 March 1982)

and

Final Report
(For the period 1 January 1965 to 30 June 1980)

by

Robert L. Squibb, Ph.D.

May 1982

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701

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Rutgers - The State University,
New Brunswick, New Jersey 08903

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PREFACE

Following notification in December 1980 of termination of this contract, all animal work was suspended on Dec. 31, 1980 and efforts then applied to finishing up the biochemical analyses of tissues and statistical analyses of the data related to the 1980 research year. A narrative report covering the period from July 1, 1980 to March 31, 1982 is included herein as the last of our Annual Reports. Another section covers a summary of the highlights of the previous 15 Annual Reports along with a list of papers published during the complete reporting period.

We have always felt that a good deal of the research on the metabolic aspects of infectious illness ignored nutritional interactions, regardless of the fact that with onset of overt illness dietary intakes are depressed. While our USAMRIID contract was specifically concerned with infectious illness, we were able to supplement the contract with outside funding. This enabled us, when indicated, to increase numbers of treatment groups during each experiment to provide simultaneous evaluation of nutritional, behavioral and/or bioclock interactions. As a consequence, in this report we are summarizing the highlights of the combined approach. The reader wishing greater detail should be advised that since 1965, 12 M.S. and Ph.D. theses were completed and are on file in the Rutgers University Library. In addition, there have been 15 Annual Reports to USAMRIID and 49 technical papers published in refereed journals; these are listed in the Bibliography.

Robert L. Squibb, Professor
Principal Investigator

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Report No. 16

Annual Progress Report

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Forced Exercise at Various Stages of an S. typhimurium Infection: Effect on Growth and Energy Metabolism

A series of trials were undertaken to observe the effect of a single bout of 2 hours of forced exercise (running wheels) administered at various stages of the S. typhimurium infection.

In the first trials, male weanling rats were inoculated with a mild dose of S. typhimurium and sub-groups were forced to exercise at either 24 or 72 hours post infection; all rats were sacrificed 72 hpi. Data on body and organ weights and liver glycogen are shown below.

Treatment	Body weight	Liver wt. Body wt.	Spleen wt. Body wt.	Liver glycogen
	g	%	%	mg/liver
-inf, - exer	98.3 ± 13.1 1/	5.6 ± 0.5	0.60 ± 0.11	227 ± 103
+inf, - exer	82.6 ± 13.5	6.8 ± 0.5	1.89 ± 0.57	37 ± 37
+inf, + exer (24 hpi)	86.4 ± 16.4	6.5 ± 0.6	1.47 ± 0.45	54 ± 72
+inf, + exer (72 ")	87.7 ± 12.8	6.6 ± 0.4	1.79 ± 0.57	32 ± 36

1/ Mean ± SEM

In the next series of trials groups of control and S. typhimurium-infected weanling rats were given a single 2-hour forced exercise session either 1, 5 or 9 days post inoculation and all were then sacrificed at the conclusion of the forced exercise period on day 9 p.i.

In non-infected controls, plasma glucose was elevated in rats exercised immediately prior to sacrifice while total liver glycogen stores were depleted 75%; there were no apparent effects on fat pad or liver lipids. However, plasma lipid data suggested that lipids were being utilized as a fuel for the energy demands of the forced exercise.

In rats forced to run 5 days p.i. and sacrificed 4 days later, liver glycogen values were repleted to values 54% higher than in non-exercising controls. Liver lipids were unchanged, while fat pad triglycerides were 26% lower in the non-exercised animals. Rats exercised 24 hpi and sacrificed 8 days afterward showed no effects of the exercise on the parameters observed, indicating the measure of repletion that had taken place.

In infected rats, immediately after exercise 9 days p.i. total liver glycogen stores were depleted 65% compared to 75% for non-infected animals. Although fat pad lipids were unaffected, liver triglycerides showed a variable response which reflected the energy demand that was not met by liver carbohydrate stores. Four days after forced exercise (5 days p.i.) total liver glycogen stores in infected rats were repleted to values 40% higher than in non-exercised infected animals. This value was less than that seen with the non-infected rats (54%). Liver lipids were unchanged, while the fat pad triglyceride values were only

15% lower. However, due to their very small size, fat pads could not be recovered in 6 of 14 of the infected animals subjected to exercise 5 days p.i.; in non-infected rats fat pads were absent in only 2 out of 16 given the single bout of forced exercise. This suggested that the mobilization of these lipid reserves either to replete other lipid stores or to provide metabolic energy occurred to a far greater extent when disease was interacted with exercise.

The spleen, a target organ, showed trends toward being smaller (by 16%) in infected rats that were exercised at 5 or 9 days p.i. This suggested that exercise may reduce the severity of the infection, possibly by stimulating the circulatory system and hence accelerating host defense mechanisms.

The data concerning these trials are to be found in the Ph.D. thesis of Gary Douglas.

Forced Exercise and S. typhimurium: Effect of Prior Training

Previous studies in these laboratories concerning the interaction between exercise and infectious illness involved the use of naive or untrained rats subjected to a single session of forced running. The energy demands of this type of exercise are met primarily by carbohydrates and, to a lesser extent, by lipids as metabolic fuel. In order to increase the involvement of lipids for fueling running exercise, and to study the effects of a series of exercise sessions on the course of a bacterial infection, a new approach for studying the interaction of exercise and disease was undertaken.

This approach involved physical conditioning of the experimental animals during the incubation period and through the onset of active involvement of the infection. Beginning one day after being inoculated with 0.5 ml (10^7 CFU/ml) IP of Salmonella typhimurium, 28-day-old male rats were put through a 6-day training regimen as follows:

Training session	Duration of exercise	Running speed
	min	m/min
Day 1	60	6
2	60	8
3	60	10
4	60	11
5	60	11
6	60	11

The day after the last conditioning session both trained and naive animals were put through a final exercise session of 2 hours @10 m/min and then all rats were sacrificed.

This approach for the study of exercise x infectious disease interactions resulted in the observation that the challenge with a bacterial infection antagonized the beneficial effects of physical conditioning noted previously.

The S. typhimurium infection resulted in a 41% decrease in liver glycogen

which was apparently used to meet the increased energy demands of the illness. Physical training resulted in increased quantities of liver glycogen of 12.5% and 65.1% in non-infected and infected rats, respectively. This increase was due to an overshoot upon repletion from the previous conditioning session. The increase in liver glycogen in the infected animals may be beneficial since liver glycogen is apparently an important energy store during infectious illness. While the relative level of repletion was greater in the infected animals, the final quantity was 14% lower than for non-infected, conditioned rats. This lower level suggests that glycogen may have been used to meet the cost of the exercise in the infected rats. Exercise depleted liver glycogen to a greater extent in untrained rats - 95% compared to 70% in non-infected, conditioned rats and 94% vs 88% for the infected animals. The training regimen resulted in a considerable sparing of hepatic glycogen in non-infected rats only. The failure of conditioning to spare glycogen in the infected animals suggests that either carbohydrate played a great role in meeting the energy demands of exercise in the infected animals, or that the presence of the illness resulted in a general increase in the energy requirement of the exercising animals, causing a greater utilization of carbohydrate stores.

Liver triglycerides (TGs) were increased 31% by the infection. Training decreased liver TGs 24% in non-infected animals but there was no effect in the infected rats. This suggests that liver TGs were used to meet the cost of training in non-infected rats, whereas in the infected animals carbohydrates served this purpose. Exercise did not cause a change in the liver TGs. The fact that the quantity of this important metabolite did not change during exercise suggests that it may remain at a steady state concentration in the liver such that as fast as it is removed for catabolism it is replaced from the plasma. This is consistent with the decrease in plasma TGs caused by exercise.

The infection caused a 25% increase in liver free fatty acids (FFAs), while training had no effect. Running exercise decreased liver FFAs 10% in naive and 16% in conditioned, non-infected rats. The FFAs did not change in untrained, infected rats but decreased 12% in trained, infected rats. These results suggest that training increases the utilization of lipids as an energy source during exercise in non-infected animals but to a lesser extent in the infected rats.

Plasma glucose was not affected by the infection or training. Exercise decreased glucose in the plasma 13% in untrained animals regardless of the presence of infection, which correlates well with the depletion of liver glycogen. Plasma glucose was unaffected by exercise in trained, non-infected animals but was decreased 16% in trained, infected rats. These changes in plasma glucose reflect the changes in liver glycogen and lipids and suggest that carbohydrate was being used as the primary energy source for meeting the demands of the exercising muscles in untrained and trained infected animals, while lipids were being used as the energy source in trained, non-infected rats.

Plasma TGs were not affected by the infection. Training did not affect the TGs in the plasma in non-infected rats but resulted in a 13% decrease in infected animals. This decrease in TGs may have been the result of uptake by

the liver since in the infected animals training did not result in a decrease in liver TGs as it did in non-infected animals. Exercise caused a decrease in plasma TGs, suggesting that lipids were being used to fuel the exercise. The decrease for non-infected animals was 51% and 47%, respectively, for untrained and trained rats. In the infected animals the decrease was somewhat less, with reductions of 38% for naive and 37% for conditioned rats. Again, this is consistent with the disease impairment of utilization of lipids as an energy source to fuel exercise.

The infection resulted in a 17% decrease in plasma FFAs. Exercise increased the FFAs in non-infected animals 30 and 40% in trained and untrained rats, respectively. A similar rise of 40% in conditioned and 44% in naive, infected animals was found. These results suggested that the bacterial infection, at the involvement level observed, did not impair mobilization of adipose stores during exercise.

Associated with the increases in plasma FFAs and decreases in TGs due to exercise was an increase in ketone bodies. Acetoacetic acid was 24% and beta-hydroxybutyrate 42% lower due to the infection in untrained animals. In trained rats the levels of these ketone bodies were apparently not affected by the infection. Training resulted in lower levels of beta-hydroxybutyrate in the plasma, with decreases of 68 and 50% for non-infected and infected rats, respectively. These results indicate that in untrained animals the infection impaired ketone body production by the liver and, in general, lipid catabolism by the liver. In the trained rats the results were less clear, but the data show a similar trend. Since extrahepatic utilization of ketone bodies was not affected by the infection, the comparable level of ketone bodies in trained animals, regardless of the presence of infection, may well be due to increased peripheral utilization during exercise. The failure of training to spare liver glycogen in the infected animals, and the effects on plasma lipids suggest that training did not alter the anti-ketogenic effect of the S. typhimurium infection.

Adipose TG stores were not mobilized by the exercise model used in these studies, nor were they affected by training. This indicates that other lipid stores must be more dynamic in response to exercise and that these stores were the source of lipid energy mobilized to fuel the exercise. The infection produced a 17% increase in the adipose/body weight ratio which correlated with an 18% increase in the TG composition of this tissue. This suggests that rather than utilize lipids as energy the infected rats were storing it in the adipocytes.

The results discussed above suggested that physical conditioning of the weanling control rats increased the efficiency with which lipid was used as a fuel and carbohydrates spared during exercise. In rats challenged with S. typhimurium, training failed to result in a sparing of carbohydrate during exercise, apparently due to an impairment of hepatic lipid catabolism as was found in naive exercised and fasted rats.

Forced Exercise and S. typhimurium: Rate of metabolic response to forced exercise

To further evaluate apparent differences in the way exercise is fueled between trained, non-infected and infected rats, it was concluded that a more complete understanding of the rates at which metabolic changes take place during exercise was necessary. Therefore, two replicate experiments were conducted under the same conditions and training regimen described above. On day 7 post infection control and infected animals were given a final exercise session of either 0, 30, 60, 90 or 120 min; rats were killed and tissues obtained immediately at the termination of the exercise.

The results are presented graphically as percent of controls (rested, non-infected) vs exercise time.

Changes in the levels of insulin and glucagon were measured as a reflection of important regulatory functions during exercise, especially a rise in plasma glucagon which stimulates hepatic gluconeogenesis. In the present study, at rest the plasma insulin concentration was elevated by the infection to 220% of control (Fig. 1), which was consistent with other studies conducted concerning infectious illness. Upon the initiation of exercise, plasma insulin levels in the infected animals were comparable to non-infected rats and remained so for the duration of the exercise session. Exercise had no significant effect on circulating levels of insulin in non-infected rats. This suggested that the infection studied did not affect the metabolic signal which suppresses secretion of insulin from the pancreas during exercise. In contrast to insulin, plasma glucagon in the controls was significantly elevated by exercise (Fig. 2), reaching 160% of the initial value by 60 min. However, the infection completely suppressed the exercise-induced rise in plasma glucagon. This suggested that the infection either blocked the metabolic signal responsible for inducing the secretion of glucagon, or prevented the synthesis and release of glucagon by the α cells of the pancreas. The probable metabolic consequence of the impairment of glucagon secretion would be a suppression of hepatic gluconeogenesis during exercise.

Carbohydrates are an important source of metabolic fuel during exercise, especially glucose. Glucose is provided to muscle from muscle glycogenolysis and the blood. The utilization of blood glucose by muscle is preferred over muscle glycogen in order to conserve the latter as a source of carbohydrate. As blood glucose is utilized by muscle it must be replenished if euglycemia is to be maintained. This is primarily a function of the liver which releases glucose produced from either glycogenolysis or gluconeogenesis. As long as the glucose output from liver is sufficient to match peripheral utilization, euglycemia is maintained. When glucose output from liver does not meet peripheral utilization hypoglycemia develops. In the present study, liver glycogen was mobilized at a fairly constant rate over the exercise intervals studied (Fig. 3). The rate of glycogen depletion was greater for the infected rats, with 88% and 58% depletion of liver glycogen after 2 hrs of exercise for infected and non-infected rats, respectively. This suggested that exercise

in the infected animals required carbohydrate to a much greater extent than in the non-infected rats. Corresponding with this greater rate of glycogen depletion was a failure to maintain blood glucose at levels equivalent to pre-exercise concentrations, compared to non-infected rats which were able to maintain euglycemia for the duration of the exercise period studied (Fig. 4). This indicated that hepatic glucose output was insufficient to meet peripheral utilization during the final 60 min of exercise in the infected animals. Of interest was the fact that the drop in blood glucose in the infected rats occurred while significant quantities of liver glycogen stores remained. This would suggest that the failure of glucose output by the livers of infected rats to match peripheral utilization was due to an impairment of gluconeogenesis due to a lack of stimulation by glucagon which, as previously discussed, failed to rise in the blood of infected rats during exercise. Thus, it would appear that the failure of infected rats to maintain euglycemia and the greater rate of hepatic glycogen depletion observed were due to a lack of glucose output by liver because of the lack of sufficient glucagon to initiate gluconeogenesis.

The metabolism of lipids during exercise augments carbohydrate metabolism and has a sparing effect on both muscle and hepatic glycogen stores. The major lipids used by muscle and liver during exercise are the non-esterified fatty acids (NEFA) which can be obtained from the lipolysis of TGs in adipose, blood, muscle and liver. The NEFA are used by muscle as a fuel for contraction and by liver for oxidation through the respiratory chain or to produce substrate for hepatic ketogenesis. In the present study, plasma TGs decreased during exercise, regardless of the presence of infection (Fig. 5). The greatest decrease was observed during the first 30-60 min of exercise, with TGs decreasing at a greater rate during the first 30 min in the non-infected rats, resulting in significantly lower TGs compared to infected rats. At the end of 60 min of exercise, however, there was no difference in plasma TGs between the two treatments. It is interesting to note that corresponding to the decrease in plasma TGs during the first 30 min of exercise was a non-significant increase in liver TGs, suggesting an uptake of TGs by the liver from plasma (Fig. 8).

Plasma NEFA significantly increased over the 120 min of exercise, regardless of the presence of infection (Fig. 6). There was a fairly rapid increase in circulating NEFA during the first 30 min of exercise, which continued in a linear fashion through 60 min for the non-infected rats only. In the infected rats plasma NEFA concentrations decreased between the exercise interval of 30-60 min, producing a significantly lower level of NEFA at 60 min compared to non-infected animals. After 60 min of exercise plasma NEFA showed a steady rise in the infected animals to levels comparable with non-infected rats. Liver NEFA were at no time significantly affected by either exercise or the infection (Fig. 7). There was an overall trend for greater quantities of liver NEFA in the infected animals. It is interesting to note that the quantities of liver NEFA did not reflect the changes in plasma NEFA in the non-infected rats, while in the infected rats the changes in plasma NEFA were directly reflected in liver NEFA during the interval of exercise ranging from 30-90 min (Figs. 6 & 7). This would suggest that

plasma NEFA were the major factor determining the quantities of hepatic NEFA in the infected rats. Liver TGs were not significantly affected by exercise in the non-infected rats but were significantly increased by exercise in the infected rats (Fig. 8).

An important relationship to note is one between plasma NEFA, liver NEFA, and liver TG in the infected rats. As already discussed, there was a good correlation between plasma NEFA and liver NEFA in the infected animals except for the first and last 30 min of exercise. During these time intervals the sharp rises in plasma NEFA were not reflected in liver NEFA. However, it was during these two time intervals that the significant increase in liver TG took place in the infected animals. This would suggest that the reason no change in hepatic NEFA occurred, while plasma NEFA showed increases, was not due to a failure of hepatic uptake but to esterification of the NEFA upon entering the liver. If this were indeed the case, one would expect to observe a decrease in the NEFA available for catabolic pathways and ketogenesis and a decrease in the rate of exercise-induced ketosis. This was exactly what was observed in the present studies (Fig. 9). During the first and last 30 min of exercise only very slight increases in the circulating concentration of plasma betahydroxybutyrate were observed compared to sharp increases in non-infected rats. In the infected rats a gradual increase in ketosis was noted during the exercise interval ranging from 30-90 min, which corresponded with the time interval in which liver TG underwent little or no change. During the same time interval in the non-infected animals ketosis did not increase any further. This suggested that during this period peripheral utilization of the ketone bodies was equal to the rate of synthesis.

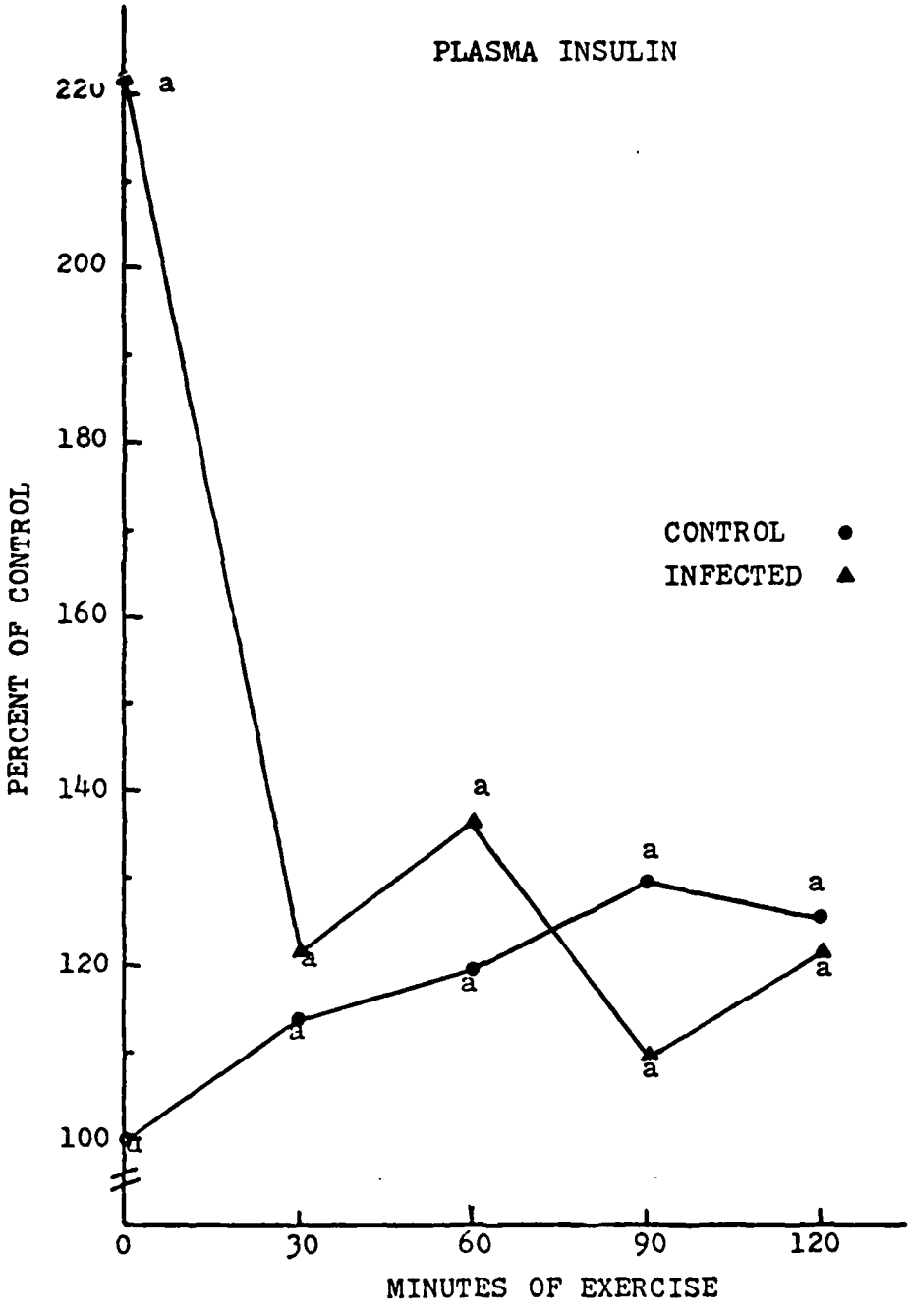
The sharp rise in plasma betahydroxybutyrate during the final 30 min of exercise in the non-infected rats suggests that exercise was beginning to become anaerobic, thus reducing the oxidation of ketone bodies by muscle; but it may also be attributed to the rising levels of plasma NEFA during this period of exercise. Hepatic monoglycerides (MG) were not affected by exercise in the non-infected rats but were increased by exercise in the infected animals (Fig. 10). The presence of the infection significantly increased the quantities of liver MG and diglycerides (DG) (Fig. 11). Exercise did not affect the quantities of hepatic DG in either infected or non-infected animals. The increases in liver MG during exercise in the infected rats further supports the hypothesis that an increase in esterification of NEFA into TG occurs during exercise in infected rats rather than catabolism, as would be expected. Failure to see similar increases during exercise in the liver DG, suggests that the formation of MG is the rate-limiting step in TG synthesis.

In summary, the present studies demonstrated an alteration in liver carbohydrate and lipid metabolism during exercise in rats infected with S. typhimurium. The infection suppressed the secretion of glucagon by the pancreas of rats in response to exercise. The failure of this hormonal response prevented the initiation of hepatic gluconeogenesis, which caused

a more rapid depletion of liver glycogen stores and hypoglycemia due to a failure of the liver glucose output to match peripheral utilization. The data further suggest that infection alters the direction of hepatic lipid metabolism away from the catabolic pathways and toward the biosynthesis of TG due to esterification of NEFA. Thus, in the infected animal glycogen stores would appear to be the major source of blood glucose for supplying carbohydrate to the muscle during exercise.

These data are presented in full in the Ph.D. thesis of Douglas Balentine.

Fig. 1.



Different letters signify statistical difference ($P<0.05$) between exercise times within treatments.

Fig. 2

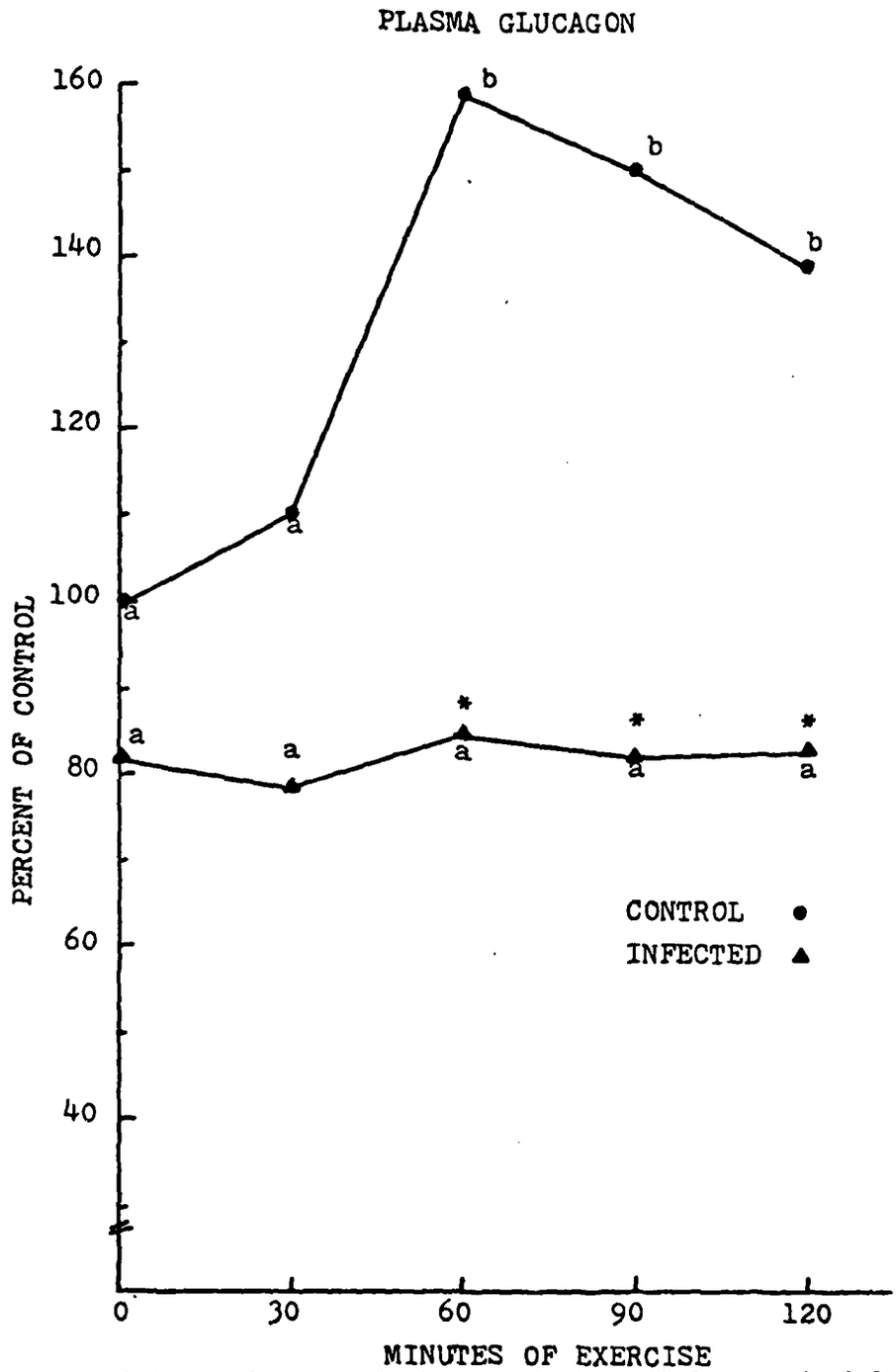


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Fig. 3

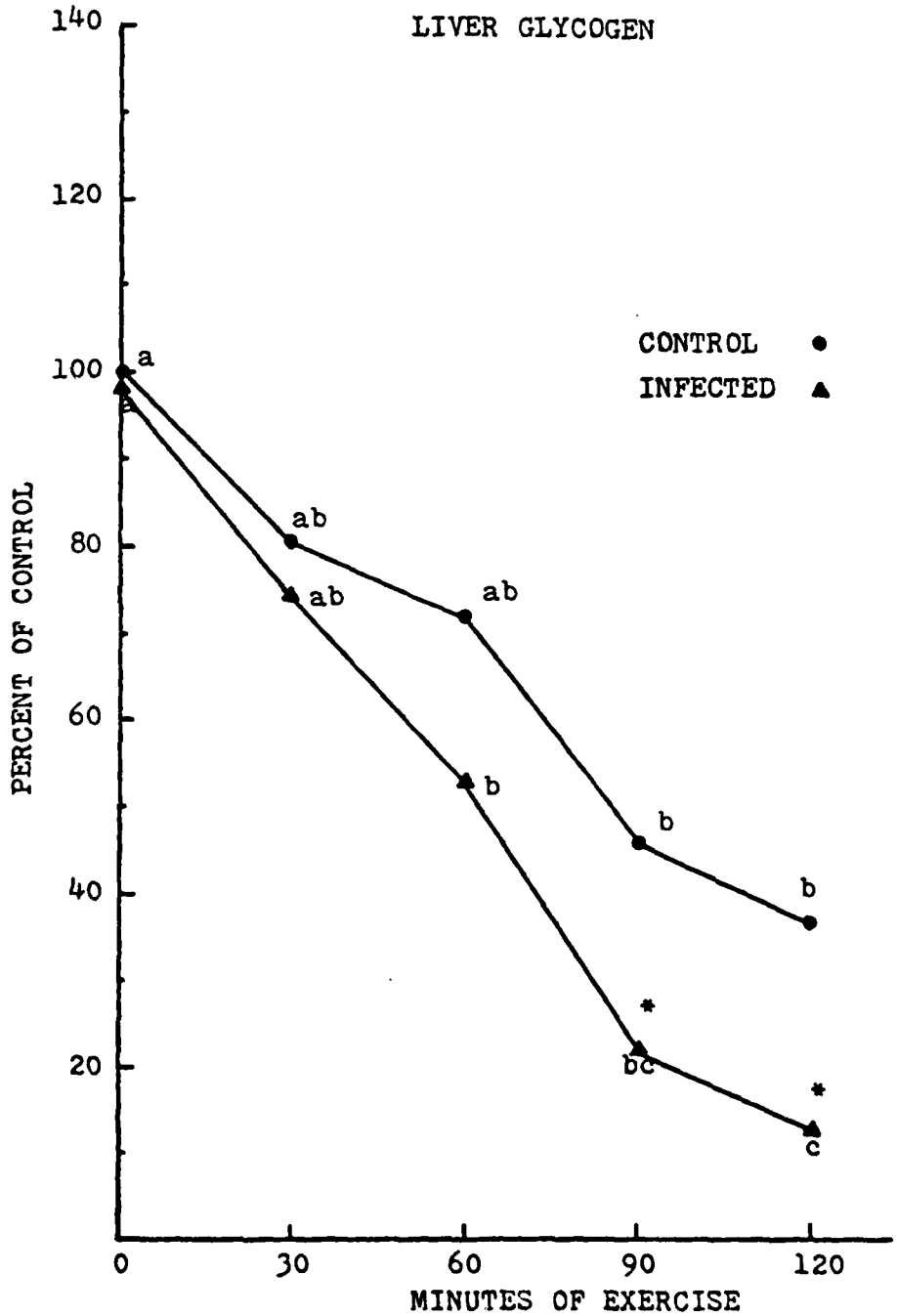

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Fig. 4

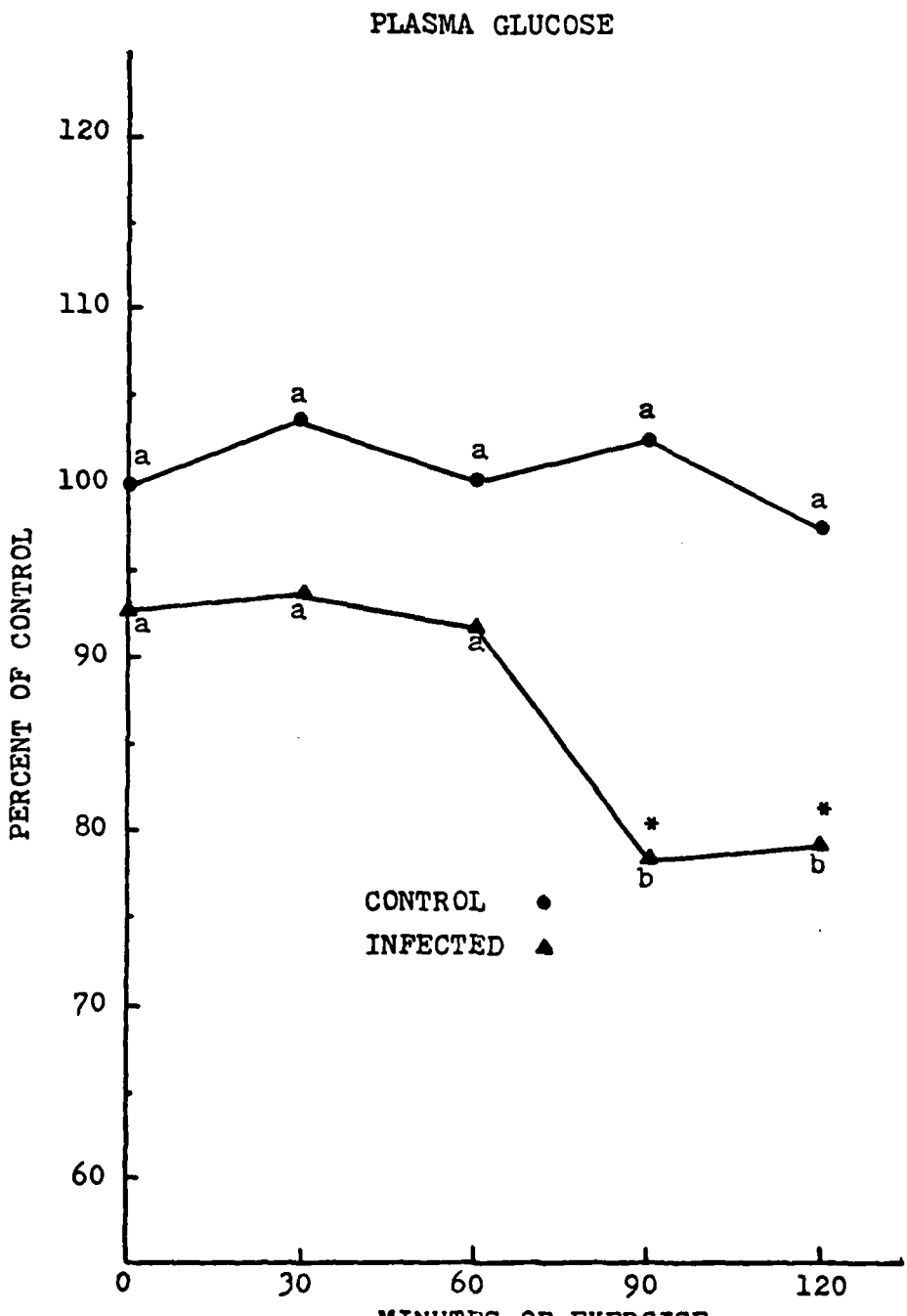

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Fig. 5.

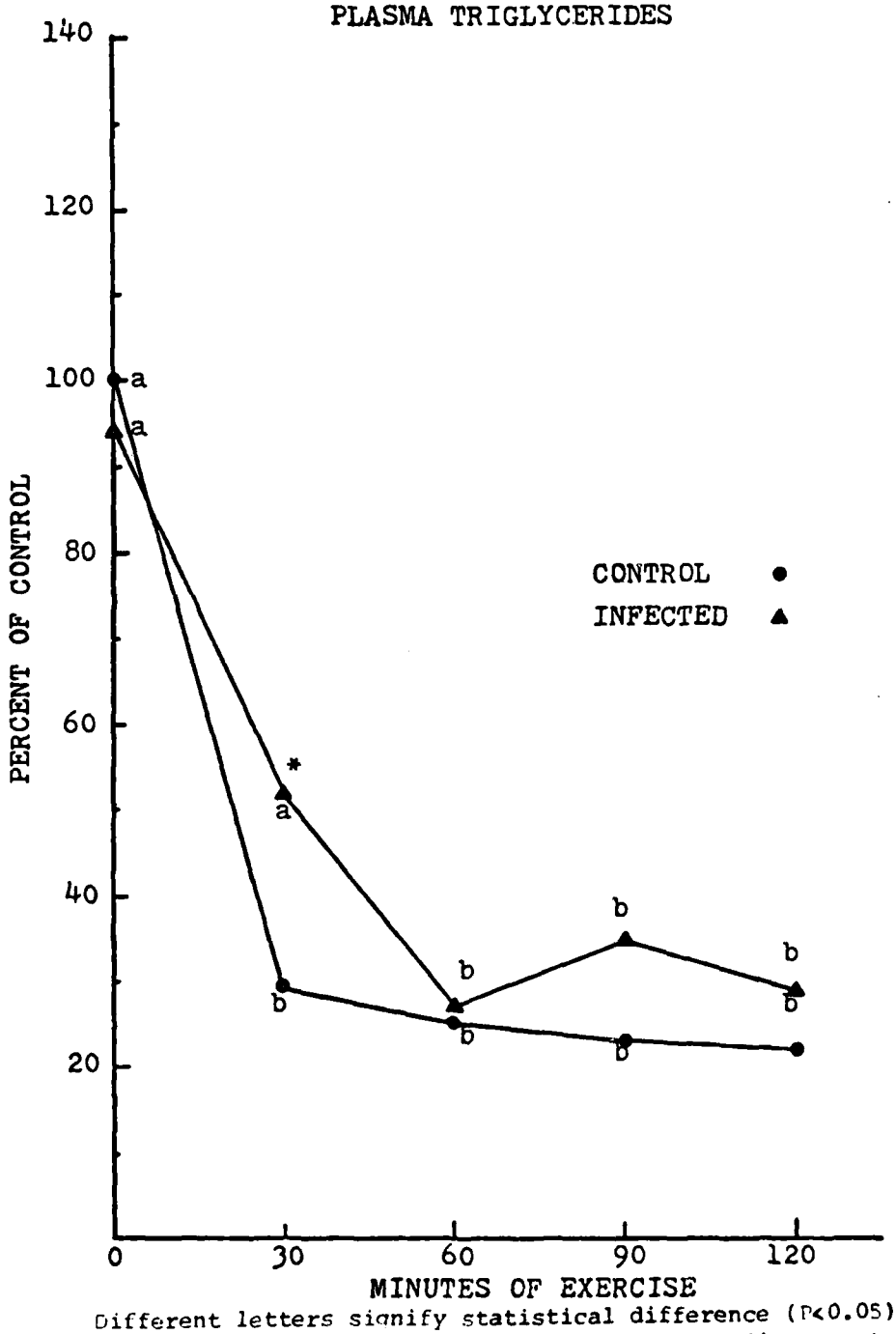

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Fig. 6.

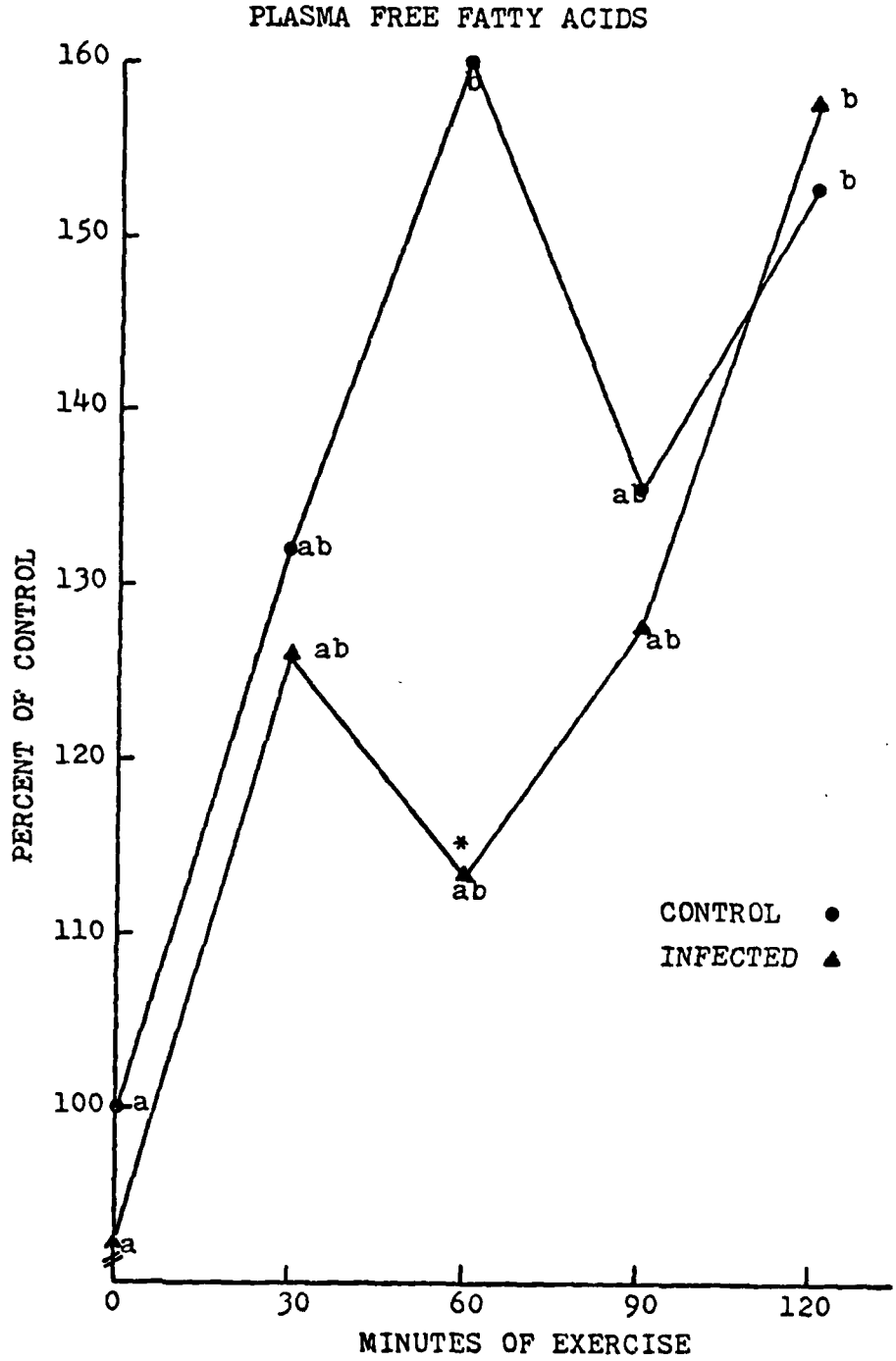

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Fig. 7

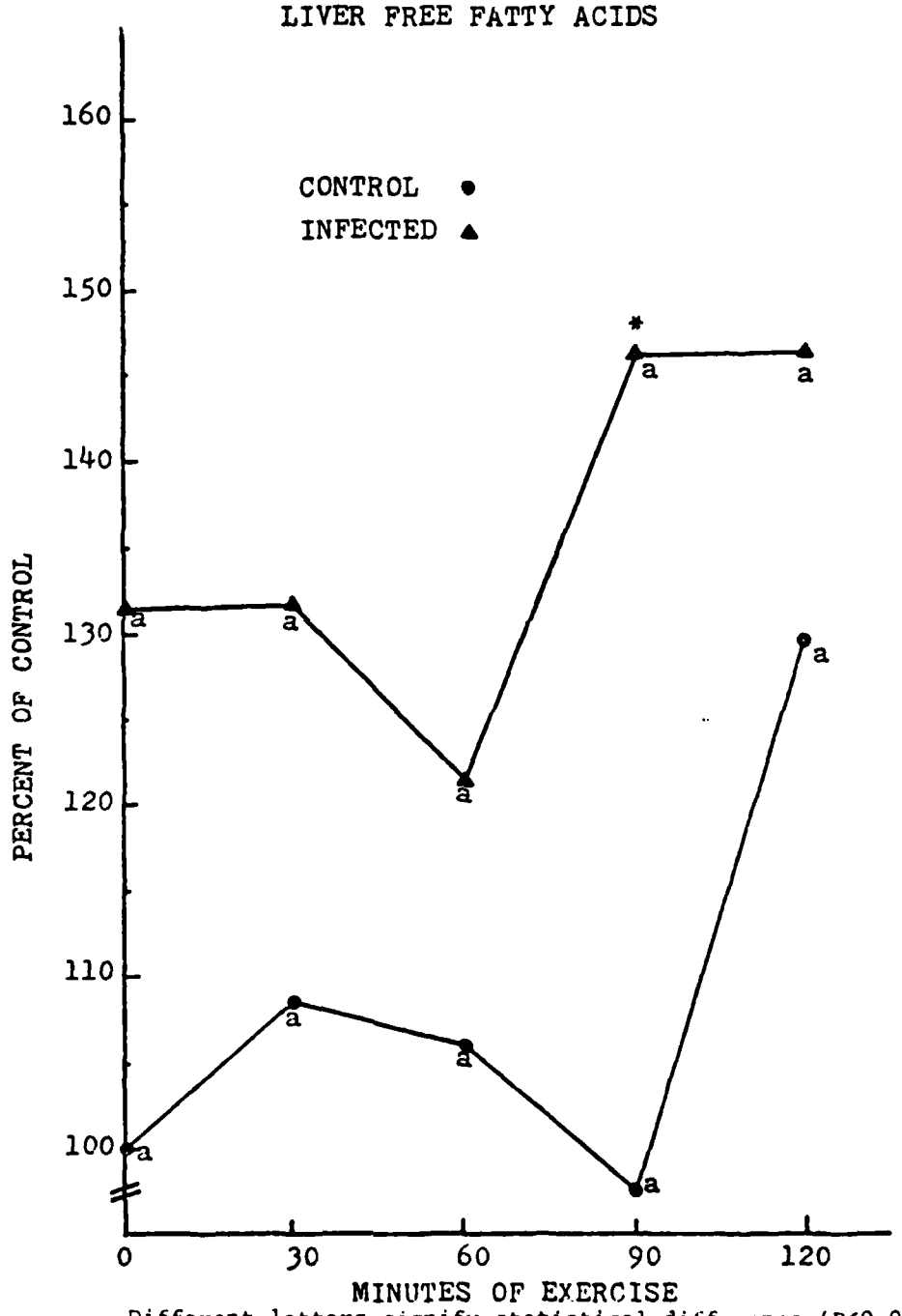

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Fig. 8

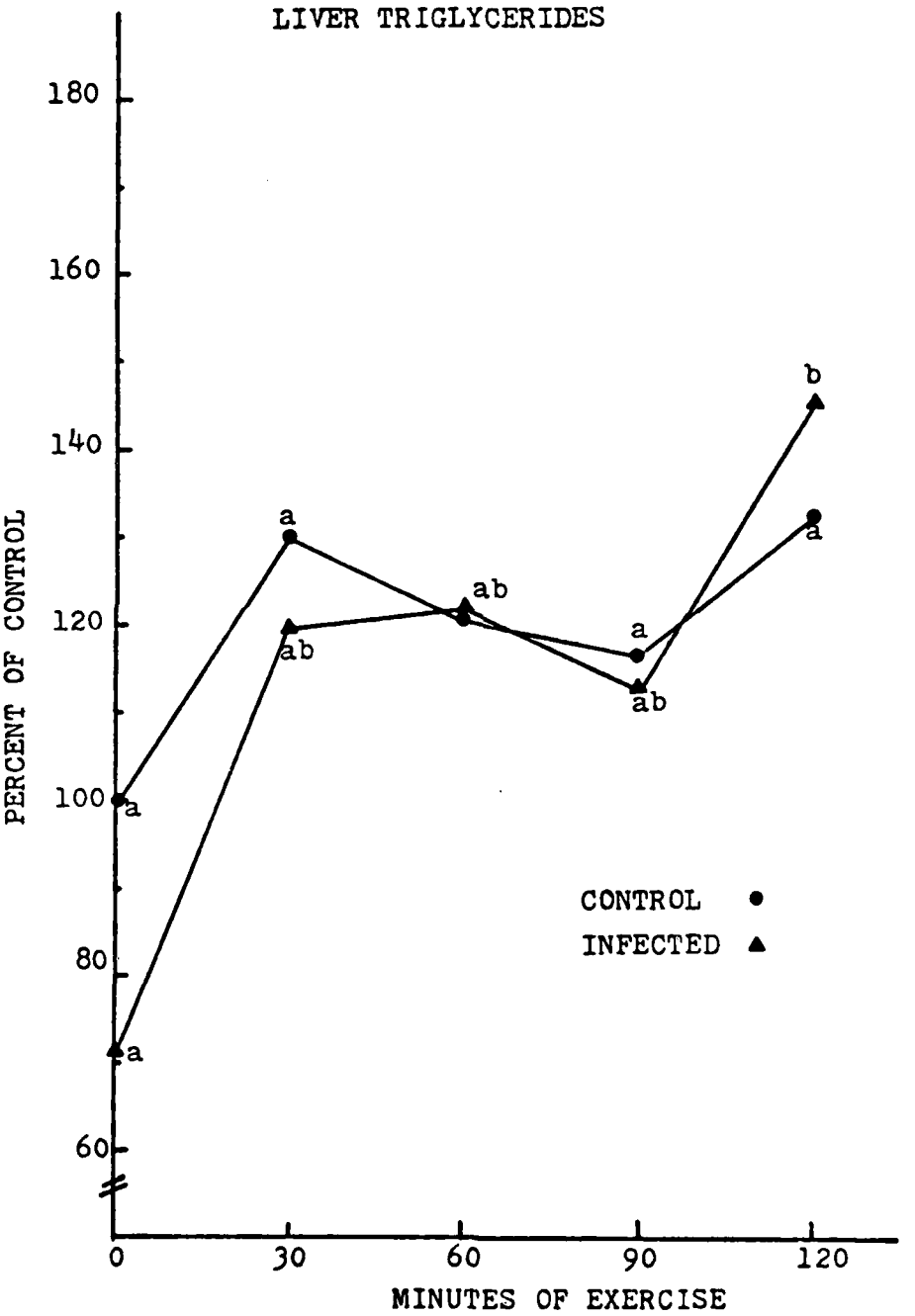

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Fig. 9

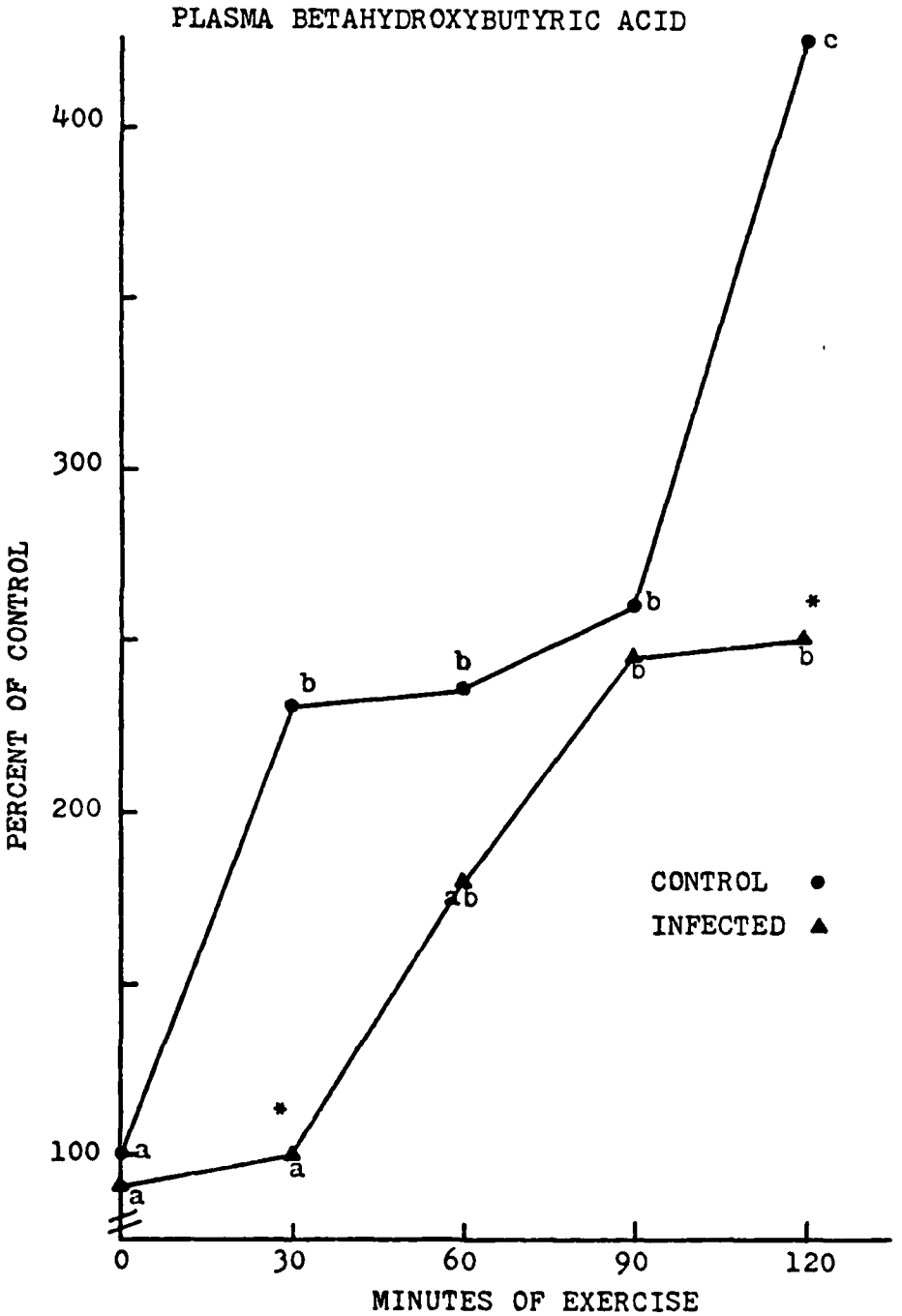

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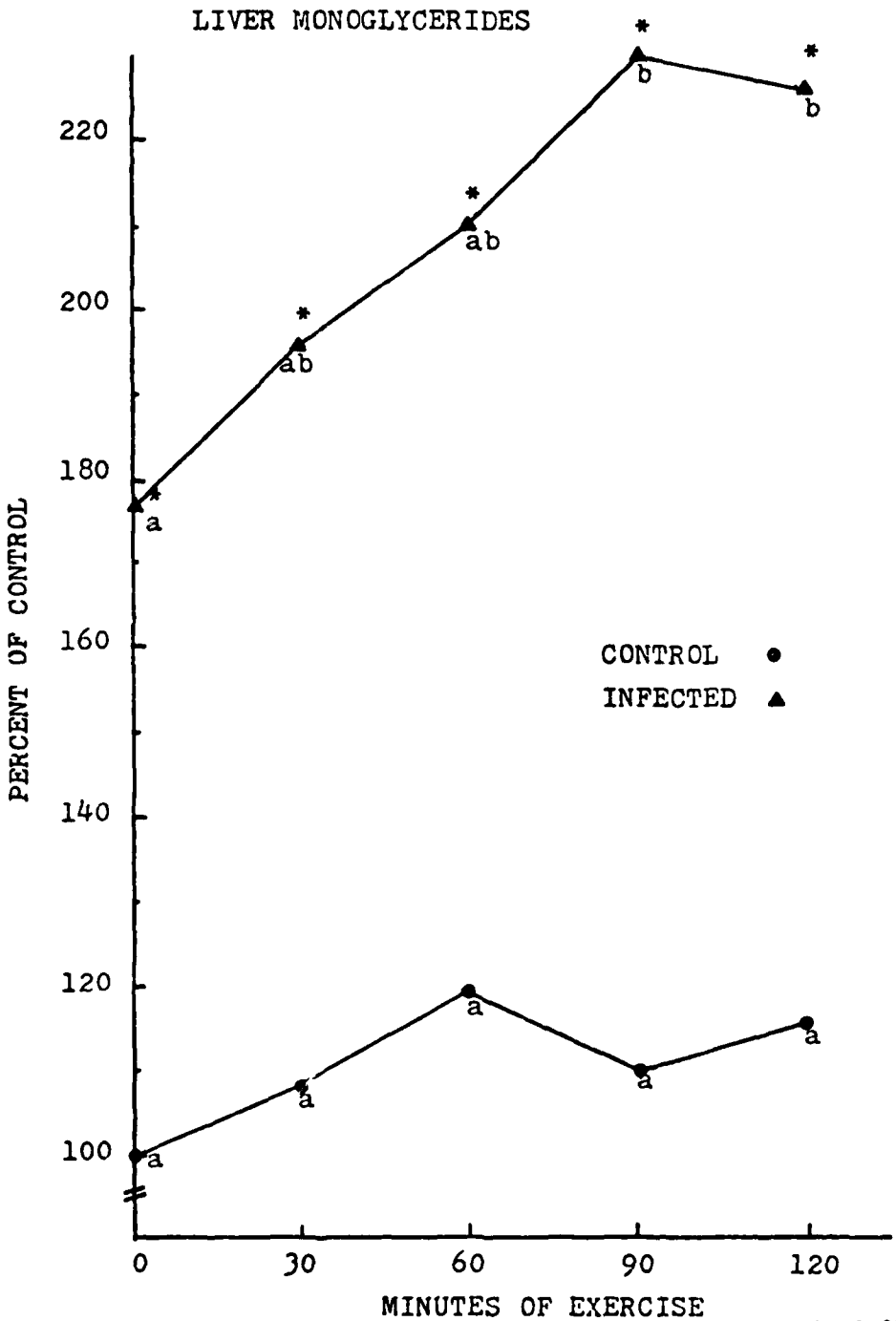
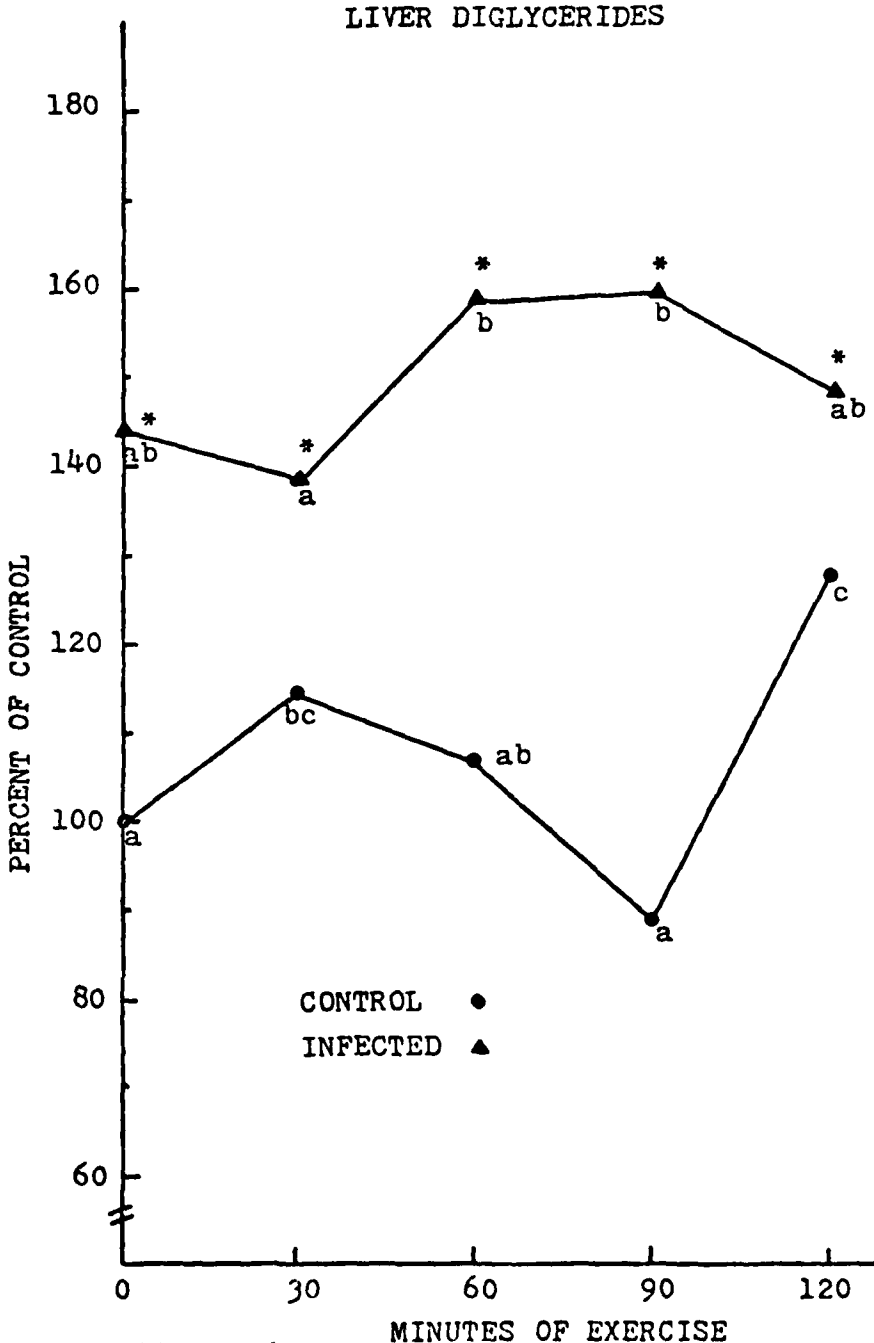
Fig. 10



Different letters signify statistical difference ($P<0.05$) between exercise times within treatments. Asterisk indicates significant difference ($P<0.05$) between infected and non-infected with respect to exercise time.

Fig. 11



Different letters signify statistical difference ($P<0.05$) between exercise times within treatments. Asterisk indicates significant difference ($P<0.05$) between infected and non-infected with respect to exercise time.

REVIEW OF WORK ACCOMPLISHED 1965-1980

1. Development of Biochemical Methods

Prior to initiation of our contract in 1965, using avian model systems, we had been studying the effects of infectious illness on vitamin and fat metabolism (1-4, 6) and on nucleic and amino acid, protein and lipid patterns in liver and other tissues (7,). The latter work confirmed the importance of studying protein synthesis and energy utilization in infected young, anabolic animals. Previously, studies on interactions of infectious illness with free amino acid pools were limited due to lack of appropriate methodology and equipment. Early on we realized that autoanalyzers could not handle large numbers of individual amino acid tissue samples which we felt were needed to obtain satisfactory statistical evaluations. As a consequence, we developed and published (8, 9) a method for the detection and rapid quantitation of some 6 to 10 amino acids in liver, plasma, muscle and other tissues. This method provided excellent precision and is still useful in 1981 despite the great progress made with automation.

2. Demonstration of the Importance of Stage of Disease Cycle and Virulence of the Infection

Early work (10, 12) confirmed the importance of carefully defining and relating sampling time to stage of a disease cycle and level of virulence of the model infection. This was called to the attention of researchers in one of our first publications on the biochemistry of survival of chicks infected with Newcastle disease virus (NDV). Repeated studies clearly demonstrated that an NDV infection resulting in from 5 to 80% mortality would have the following cycle: 72 hr incubation (no effect on food consumption or weight gains); 72 hr of active involvement or overt illness during which the major mortality would occur and feed intake would be depressed, accompanied by loss in body weight; and finally, 72 hr of what we termed "initiation of recovery." In survivors food intake and body weight would increase, mortality would cease; however, depending upon the virulence of the infection, irreversible paralysis could occur. Birds sacrificed at specific intervals during a disease cycle would show significantly different biochemical effects. This was most evident from our studies (5) of the effect of NDV on nitrogen retention in susceptible chicks. Nitrogen retention increased significantly during the incubation stage of an NDV infection having a reference end point mortality of 35%. The depressing effect of the infection on diet intake confounded the disease effect per se and resulted in large losses of liver nitrogen during overt illness. This phenomenon was reversed by "catch up" retention during initiation of recovery from the infection. Of particular interest in these studies was that when an immunizing level of infection was studied nitrogen retention continued high throughout the entire 216 hr of the NDV cycle. This increase in nitrogen retention correlated with a rise of HI titer. It was postulated that this increase in nitrogen retention was part of the defense mechanism.

3. Effect of Dietary Protein on Infectious Illness

While in 1965 there was considerable evidence in the literature that dietary protein could significantly alter the course of an infection, little work had been reported on biochemical changes associated with the observed deteriorating clinical picture and increases in mortality. As a consequence, we conditioned cockerel chicks (11) with diets wherein protein content ranged from deficient, balanced and imbalanced to surfeit and infected them with NDV at 28 days of age. Liver DNA averaged higher in the surfeit protein groups. RNA, protein and free amino acids in terms of DNA were highest in the birds provided normal levels of dietary protein. Based on the data observed, it was postulated that protein metabolism was most efficient in birds provided dietary protein in amounts normally assumed to be within requirement. Highest mortality was observed in the deficient and surfeit groups. This was attributed to double jeopardy, e.g., a possible failure of kidney function during elimination of protein-associated metabolites. Significantly lower HI titers correlated with extreme protein deficiency. Most interesting was the fact that mortality was independent of HI antibody titers and showed no apparent correlation with liver nucleic and free amino acid levels.

Two later studies (13, 20) further confirmed the adverse effects of deficient or surfeit quantities of dietary protein and lysine on protein metabolism. In chicks conditioned on deficient-to-excess quantities of protein and/or l-lysine and infected with a bacterial model (Mycobacterium avium), liver size (target organ) increased significantly with the intensity of the infection, which was manipulated by using different TB strains and/or varying the numbers of organisms injected. Amino acids decreased as liver size and the degree of involvement of the infection increased, with lysine and arginine showing the greatest depression. Dietary intervention, e.g., feeding deficient-to-excess quantities of crude protein or crystalline l-lysine resulted in least involvement in the groups fed normal levels of these dietary essentials. Lysine absorption studies (20) in noninfected chicks clearly demonstrated that as levels of dietary lysine increased from deficient to surfeit the ratios of free amino acids in intestine and liver changed, correlating with significant depressions of leucine, arginine, histidine and valine in the serum.

4. Relation of Clinical Symptoms to Biochemical Changes in Liver

Another area of research that was in progress and later supported in part by USAMRIID were studies pertaining to the status of liver nucleic and free amino acids in relation to clinical symptoms and the anorexia produced by infectious illness (10). Clinical symptoms of NDV arrayed in order of increasing involvement correlated with a linear depression of body weight and an increase in liver size (previously observed to correlate with a greater retention of nitrogen (5). These data were accompanied by linear increases in liver DNA concentration and decreases in RNA, total protein and free amino acids, demonstrating the increasing depressive effect of infectious illness on protein synthesis in a rapidly growing (anabolic) chick which more than doubles its body weight the first 7 days of life. During the period of overt illness (5 days p.i.) anorexia resulting from the NDV was more significant than the illness per se in its effect on liver nucleic acid values. The free amino acids, on the other hand, were affected by both the disease and the anorexia, indicating the potential that changes in amino acid levels might have for the early detection of disease. During recovery the nucleic acids and tissue protein returned to normal values

or reflected the increase of protein synthesis as it related to "catch up" growth. Further depressions of the amino acid building blocks correlated with the growth phenomena.

Alkaline phosphatase tissue levels observed in chicks infected with NDV differed from those observed in mice. In the latter, AP activity increased in the small intestine and serum, but decreased in liver during D. pneumoniae infection. In NDV-infected chicks the reverse was observed, AP increasing in liver and decreasing in serum and small intestine. These changes occurred prior to onset of overt illness, which would indicate they were due to the infection per se rather than secondary metabolic changes (18).

5. Biorhythms

Our first evidence of biochronological interactions with infectious illness was obtained when we sampled control and NDV-infected birds sequentially around-the-clock. Analyses of liver protein, nucleic and amino acids, all parameters of protein synthesis in the young (anabolic) chick, revealed that these parameters fluctuated in both control and infected birds and that these changes conformed to clock hours (12). In other words, we had encountered the phenomenon of circadian rhythmicity which because of its magnitude had to be considered in all future designs to avoid confounding. Later (24) we observed that there were definite rhythmicities of within group variability, e.g., the standard deviations of the means, that could be influenced by Zeitgeber inputs. These data were important because where genetic constitution may be influenced by treatment, part of the effect may be related to a shift in circadian timing within living intact systems.

For the next several years generally we used the NDV infection of chicks as the model system to define the extent of error circadian rhythms might contribute to early diagnosis of infectious illness. Large groups of susceptible chicks, inoculated with varying intensities of NDV, were sacrificed in groups of 8-10 at regularly spaced intervals at specific stages of the NDV disease cycle. Diurnal changes in protein metabolism were studied (16) over the 72-hr incubation period of the NDV. Protein, DNA, RNA and free amino acid levels were determined, where applicable, in liver, muscle and serum. The data were plotted in relation to clock hours. Significant diurnal rhythms in all three tissues confirmed the presence of periodicity. In the controls, liver DNA was highest in the evening but maximal values of RNA, protein and the free amino acids occurred during the day. While the free amino acid pool in the liver was significantly depressed by the NDV, in the serum there was an increase in values attributed to "back up" and/or mobilization. The NDV also caused a significant desynchronization in relation to clock hours of the rhythmic patterns in the liver and to a lesser extent in muscle. The effect in the liver was noted within 12 hr p.i. In general, the data demonstrated that diurnal rhythms of various tissue constituents can occur with considerable magnitude. Periodicity phenomena therefore must be recognized and considered in the diagnosis of infectious illness.

6. The free amino acid pools and infectious illness

The need to better understand the effect of infectious illness on the free amino acid pool(s) in liver and serum of chicks during the initial stage of an NDV infection was accomplished in part by sampling large numbers of chicks at spaced intervals during the 72-hr incubation period of the NDV (15). In all, 7 free amino acids were determined and the ratic of each amino acid to the total for the 7 were calculated.

There were significant changes in free amino acid pool size in control and infected livers which were related to sampling time and to the NDV. In spite of these fluctuations in pool size, there was a remarkable constancy in the percentage of each of the individual components. For example, while pool size for the controls changed as much as 40%, the value for lysine remained between 10 and 12% of the pool. However, even these slight changes were significant. Variability in pool size was greater between chicks within sampling periods than variability in percentage of the individual amino acids in the fluctuating pools. Except for the first sampling period 7 hpi, pool sizes of the control and infected groups showed significant divergence at certain sampling hours. The daily average of the pool size over the 72-h period in the controls showed no trend, whereas in the infected groups there was a linear decline.

Similar data were observed in the sera of the control and infected chicks. The curve of the infected groups was slightly higher than controls but fluctuations in both curves were related to sampling time and not to the NDV. As in the liver, the percentage of the individual amino acids in relation to the total of the 7 determined was remarkably constant.

The data were evidence that most phases of metabolism are maintained in balance by a homeostatic mechanism(s). This would be expected unless unusual stress occurs, since the free amino acids reflect the average of the various pools which provide the prima materia to the different codes governing the synthesis of proteins.

7. The Effect of Infectious Illness on Serum Copper, Zinc, Cholesterol and Carotenoids

The fact that tissue constituents other than those related to protein synthesis fluctuate significantly was shown in a 9-day around-the-clock study of changes in serum copper, zinc, cholesterol and carotenoids in chicks infected with NDV (29). The data indicated that an NDV infection (48% mortality) was associated with increases in serum copper and cholesterol and depressed values for serum zinc and carotenoids. During the NDV incubation period (0 to 72 hr) the depression in zinc and increase in copper concentrations, and to a lesser extent total cholesterol, were true infection-related effects since dietary intake of the chicks remained normal at this time. An observed depression of total serum carotenoids, on the other hand, could have been influenced by the reduction of food intake which began 72 hpi.

The study suggested that infection-related influences on both periodic rhythms and on deviations from a normal range of concentrations may vary from substance to substance during the same time period in a single infection. The data also emphasized the influence of periodicity. The diurnal oscillations observed had a large magnitude of change, e.g., serum copper varied as much

as 75% in 24 hr, to illustrate the size of potential error. If the concentration of a substance normally undergoes rhythmic changes during the course of an investigation, a set of values obtained at a single point in time cannot be employed as an acceptable control sample. Moreover, the diurnal patterns of the oscillations indicated that serum copper in normal chicks possessed a classical circadian rhythm; yet in the same milieu at the same time zinc, carotenoids and cholesterol apparently lacked a definite pattern.

8. Effect of Infectious Illness on Protein Synthesis in Cardiac Tissue

Early studies on protein synthesis in the hearts of rats and chicks established that DNA was not necessarily a cellular constant and that this vital process was highly sensitive to viral invasion. The first report (17) observed nucleotide rhythms in the mature rat heart. The finding of circadian rhythms of DNA in an organ known not to have cellular division was attributed to absolute changes in quantities of this nucleic acid. A diurnal rhythm which is the result of an increased synthesis of DNA is apparently unrelated to the production of new cardiac cells. Other data demonstrated that the free amino acid pool was highly dynamic in this vital organ, as evident by changes reaching 40% within a 24-hr period. Changing laboratory lighting regimens from 12 hr light/dark to 24 hr light inverted DNA and RNA biorhythms in the chick heart (27). An NDV viral challenge adversely affected protein synthesis in the chick heart (22), as evidenced by significant depressions of heart size, synthesis of cardiac protein (DNA, RNA and free amino acids), all of which occurred in the very early stages of the infection, e.g., during the incubation stage of the NDV.

9. Interaction of Lighting Regimen and Infectious Illness: Effect on Hepatic Protein Metabolism

The interrelationships of lighting regimen and NDV infection to diurnal rhythms in liver components associated with protein metabolism in chicks were observed (21). Progressive within-day sampling of tissues of chicks conditioned to a 12 hr light/dark (12 LD) or constant (24L) schedule and infected with NDV showed significant differences in diurnal patterns the first 72 hrs post infection, the incubation period of the NDV.

In the 24L groups liver weights of control and NDV chicks increased linearly but the increase was significantly higher in the infected birds. These data confirmed our previously reported increase in nitrogen retention (5) during onset of infectious illness. Under the 12LD schedule both control and NDV liver weights had diurnal rhythms with troughs at 0800 hr, but beginning 40 hpi the infected livers increased significantly above controls. In NDV chicks under 24L, DNA concentrations were desynchronized compared to control patterns during the 72-hr observation period. With the 12LD regimen liver DNA patterns in the infected chicks were similar to controls the first 12 hpi but in the next 19 hr DNA levels were decreased and the patterns desynchronized; after this interval the oscillations resynchronized with controls. RNA and free amino acid values were significantly depressed by the NDV for most of the 72 hr incubation period in chicks under the 24L schedule. With the 12LD schedule RNA levels and patterns were similar for control and infected chicks, as were the free amino acids.

The data showed that the effects of the NDV on cellular constituents of the

liver were more pronounced under the 24L regimen. This was thought to be due to double jeopardy - the stress of the constant light plus the stress of the NDV.

10. Effect of Lighting Regimen on Diurnal Distribution of Amino Acids Between Blood Cells and Plasma

The phenomenon of the distribution of amino acids between chick blood cells and plasma was investigated (28) under two lighting schedules: constant (24L) and 12 hrs light/dark (12LD). Blood samples were collected from non-infected chicks at 6 spaced intervals over a 24-hr period. The blood cells were separated from the plasma and both were analyzed for lysine, histidine, arginine, aspartic acid, alanine, valine and the leucines.

The data demonstrated that there was a dynamic movement of the free amino acids between the tissues. For example, plasma-to-cell ratios ranged from less than 1 for lysine to greater than 1 for the other amino acids determined. Calculating percentage change of diurnal concentrations of amino acids in cells with plasma values equated to 100 revealed a very definite rhythmic exchange of these building blocks between the avian nucleated cell and plasma carrier. Further, it was evident that the uniform rhythmic patterns established by the 12LD regimen occurred over approximately 15 hrs rather than 24 and were selectively altered by changing to 24L. The fact that histidine patterns, and to a lesser extent value, were similar under both regimens permits the speculation that lighting was specific in its reaction and that diet intake was not confounding the results. This was further confirmed by the fact that similar feeding patterns were observed between the two lighting groups. Since the diurnal changes in concentrations of amino acids were sensitive to light schedule, there remains the possibility that other unknown Zeitgeber inputs could be equally confounding.

11. Effect of Lighting Regimen on Diurnal Aspects of Protein Metabolism in Heart, Intestine, Pancreas and Liver

Using the same experimental design as in Item 10 above, the effects of lighting schedule on biorhythms were investigated in heart, intestine and pancreas as well as livers of noninfected chicks.

Patterns of DNA oscillations in the liver and heart under 24L were similar and quite different from those in the intestine and pancreas. The 12LD regimen tended to invert the liver and heart rhythms, change the intestine pattern altogether, but did not affect the oscillations in the pancreas. Peaks and troughs of RNA in the liver resembled the DNA rhythm but the other RNA curves were irregular under both lighting regimens. In the liver the protein fluctuations were similar regardless of lighting schedule; in the intestine and pancreas the patterns were inverted, while in the heart there was no similarity. Total free amino acids showed similar troughs in the intestine but no similarities were evident for the other tissues.

Patterns for lysine were inverted by lighting schedule in the liver, irregular in the heart and pancreas and comparable for the intestine. Liver arginine was inverted by lighting, irregular in the heart and reasonably similar in the intestine and pancreas. Valine patterns were irregular in the liver and

heart and inverted in the intestine and pancreas. Leucines were also irregular in the liver and heart, similar in the intestine and inverted in the pancreas.

The data clearly demonstrated that lighting schedule could affect diurnal patterns of tissue constituents, a phenomenon which not only illustrates the dynamics of the intact system but also poses problems of interpretation of data when tissues are sampled at a single point in time.

12. Dynamics of Energy Components of Serum, Liver, Heart, Pancreas, Intestine, Spleen and Muscle

Most of our early studies were concerned with the interaction of infectious illness with protein synthetic processes. Investigations into the contribution of energy to the course of an infectious illness were now indicated. The following experiment was conducted to obtain baseline data. Again, our chick model and a 12LD lighting system were used and the birds were sacrificed at spaced intervals over a 24-hr period; all chicks were noninfected. Serum, liver, heart, pancreas, spleen, intestine and breast muscle were obtained and analyzed individually for glucose, glycogen, free fatty acids, mono-, di-, and triglycerides and cholesterol fractions

Body mass and liver weights reached maximum at 2000 hrs, the end of the light period and cessation of feed intake (chicks did not eat in the dark period). Since water comprises the largest percentage of the body mass and its individual tissues, the livers were taken to dryness to determine the influence of this vital fluid on the observed diurnal rhythmicity of liver weight. The moisture content of the livers was found to be 76.4% at 0800 hrs; 76.3 at 1500; 74.5 at 2000; 76.1 at 2400; and 77.2% at the second 0800 hr sampling period. These changes, having a range of 2.7% were significant and were in inverse relationship to the changes in weight of the whole liver. Unpublished data of our laboratories indicate that the carbohydrate and protein fractions account for the major part of the observed diurnal variability in liver weight.

Of the tissues observed, the greatest diurnal changes occurred in serum, liver, pancreas and spleen. Glucose, glycogen and the glycerides were the most dynamic. Even though present in small quantities, hepatic glucose levels rose 70% from 0800 to 1500 hrs; glycogen, which makes up the principal carbohydrate in the liver, increased by 150% in the same period . Liver di- and triglycerides also fluctuated considerably - 30 and 50%, respectively. In the serum the glyceride values dropped between 30 and 50% from 1500 to 2400 hrs. In the pancreas these energy components reached maxima at 1500 hrs and minima at 2000, rising again at 2400 hrs. In the spleen the mono- and triglycerides reached a peak at 2400 hrs while the peak of the diglycerides occurred at 1500 hrs. In the intestine and muscle the glycerides all fluctuated with the same pattern but with no appreciable magnitude. For free cholesterol and cholesterol esters the greatest diurnal variation occurred in the spleen where the curves were highest at 2400 hrs.

It was expected that these data would provide an insight into the dynamics of simultaneous changes in seven important tissues. Because of the magnitude of change observed in liver glucose, and especially glycogen, it was evident that where these energy components are under study great care must be taken in choosing the proper sampling time.

13. Energy Costs of a Tuberculosis Infection

The next series of studies was undertaken to explore the possibility of estimating the energy demands of a known level of infectious illness. The need to obtain individual data from large numbers of animals and to use both bacterial and viral model infections posed complications of cost, space and equipment. As a consequence, we developed methodology which was based upon a new procedure (26) we had recently established for rapidly estimating the metabolizable energy of foodstuffs. This method employs 9-day-old chicks. Energy demand of a stressor can be calculated by interacting an infectious illness or other stressor with the known caloric contribution of 10% corn oil which is 9 kcal/g. If in the presence of a disease stress the kcal yield drops, for example, to 4.5 kcal/g, then it is assumed that 50% of the calories have been utilized by the infection.

Three trials related to the bioenergetics of a tuberculosis infection in chicks were conducted (30). Inocula were adjusted to yield mild or severe involvements. Trial 1 (mild) indicated that 6% and trials 2 and 3 (severe) 96 and 93% of the energy supplied by corn oil in the test diet to fuel growth were utilized instead by the tuberculosis process. In the birds given the low level of inoculum, the degree of tuberculosis involvement, as measured by increased liver size, was correlated with increased total quantities of hepatic RNA, monoglycerides, free fatty acids, free cholesterol and glucose. All of these effects were observed prior to manifestations of clinical symptoms or failure of the chicks to consume all food offered, thus avoiding dietary confounding.

14. Energy Costs of Infectious Illness and Exercise

Further studies with this energy model enabled us, for what we believe to be the first time, to calculate the daily costs (kcal) of disease x exercise interactions. Both mild and virulent levels of NDV were interacted in the chick with voluntary access to running wheels and the dietary utilization of food energy calculated in individual animals during the incubation stage of the NDV. Energy costs were determined from growth equivalents (GE). The mild NDV infection increased growth over noninfected controls. This phenomenon was interpreted as resulting from an increased retention of essential nutrients (31). This increased retention of nutrients during the early stage of an infection increased the availability of calories for growth. Conversely, for the severe infection, voluntary wheel running and the NDV interacted, with the NDV lowering GE values by 30% or 44 kcals; voluntary exercise, in turn, reduced GE values in controls 40% (86 kcal) and NDV x exercise 52% or 112 kcal.

15.0 Effects of Interactions of Infectious Illness, Exercise and Diet on Physical Well-Being

Starting in 1977, our objective turned to trying to elucidate the effects of interactions of infectious illness, exercise and diet. The rationale was that combat readiness requires that troops be in top physical condition. Ability to move quickly and over long distances for indefinite periods of time is necessary for successful operations. The importance of understanding such interactions is vital, yet present knowledge in the area is inadequate. It became obvious that our contribution could be made through development of animal models which would permit basic observations not possible in human subjects.

Before starting the exercise research it was necessary to 1) define exercise, and 2) design, construct and test the necessary apparatus to obtain valid data. Our working definition of exercise was to divide the term into three categories, namely: forced (command), voluntary (spontaneous activity), and lastly, alarm reaction or response. These categories apply equally to humans and animals. Voluntary activity and alarm response can be measured by housing rats or chicks in standard Wahmann running wheels. But to obtain data on forced exercise, Wahmann running wheels were modified with pulleys aligned in series to a single shaft driven by a G.E. motor adjustable from 0 to 100 rpm. The apparatus was unique in that rats or young chicks or a combination of both could be run simultaneously. A single caretaker could easily control up to 18 animals run individually or 36 when run in pairs (which proved to be very successful). The wheels were easily disassembled at the end of an experiment so that they could be sterilized before another trial.

More than 30 experiments wherein infectious illness, forced or voluntary exercise and energy source were studied in one, two, or three-way interactions. Both weanling male rats and day-old male chicks were used to observe species differences. Challenges with infectious illness, e.g., S. typhimurium for rats and avian tuberculosis for chicks, were considered low level. Maximum forced exercise period was a 2-hour session at 12 rpm. Nutritional sub-plots were included which permitted comparisons of glucose, fructose or sucrose as major sources of dietary energy; natural and synthetic energy sources were also compared. Animals were sacrificed and various tissues analyzed for lipid fractions, glucose, fructose, glycogen, ketone bodies, nucleic acids and enzymes involved in protein synthesis. The data are presented in full in the Master's or Ph.D. theses listed in this report.

15.1 Forced Exercise and Stage of the Disease Cycle; Effect on Mortality

In two trials rats were inoculated with either a dose of S. typhimurium to yield 50% mortality (trial 1) or a dose twice as high (trial 2). Twenty-four hours post inoculation half the rats in each trial were forced to run for 75 min and then given no further exercise for the duration of the 12-day trials. In trial 1, first death occurred on day 7 in the rats forced to run and on day 10 in the non-exercised group. By the end of 12 days 17% of the non-exercised rats

had died compared to 38% in those forced to run 24 hpi. Trial 2 was concluded on day 6 when all rats had died; the level of disease involvement had masked any exercise effects. Growth of the forced-run rats that lived was identical to the non-exercised rats until day 4 when there was a decrease for the rest of the observation period, even though the animals had been forced to exercise for a single time period - 24 hpi - during the course of the infection. (See Ph.D. thesis of Gary Douglas)

15.2 Forced vs Voluntary Exercise

Observations were made between rats forced to exercise in running wheels for 5 min/day for 7 days and then given a 2-hour exercise session before sacrifice vs rats allowed voluntary access to wheels whenever the others were forced to run. During the final exercise session the rats forced to run lost twice as much body weight as the rats running voluntarily, even though the number of wheel turns for the latter were 28% more. Compared to non-exercising rats, liver lipid fractions were increased under both exercise conditions. Later trials comparing the same exercise protocols but including rats infected with S. typhimurium produced similar data with respect to the non-infected groups. However, the effects of the S. typhimurium on liver lipid fractions were less when the infection was interacted with either voluntary or forced exercise. These data appear in the Master's thesis of Herbert Rudolph.

15.3 Natural vs Synthetic Energy Sources x Infectious Disease x Exercise

Weanling rats were inoculated with a mild S. typhimurium infection, allowed constant voluntary access to running wheels, and fed either a diet containing a natural energy source (corn oil) or one with synthetic energy (1,3-butanediol, 1,3-dioctanoate - BDDO). The non-infected rats fed the BDDO ran more than those fed corn oil, but in the infected groups there was no diet effect. The infection increased liver size and liver/body weight ratios in non-exercised and exercising rats. In the corn oil-fed rats, compared to those fed BDDO, there were more disease effects in the non-exercising animals: hepatic RNA, protein, lysine, histidine, aspartic acid, valine, the leucines, monoglycerides and cholesterol were increased; in the exercising rats the only disease effects were in hepatic RNA, protein, histidine, the leucines and cholesterol. When the rats were fed BDDO, except for increased RNA, the disease had little significant effect on liver composition. These data demonstrated an important interaction between disease, exercise and energy source which was hypothesized to be due to differences in metabolic pathways. The data appear in the Ph.D. thesis of Herbert Ruttenberg.

15.4 S. typhimurium, Forced Exercise and Dietary Energy: Effect on Constituents of the Weanling Rat Heart

Control and S. typhimurium-infected rats (weanlings) were fed diets containing either 45% sucrose or fructose as the major energy

source. At 9 days post infection sub-groups of rats were forced to exercise 2 hours in running wheels and then all rats were immediately sacrificed. In general, dietary fructose decreased heart weights regardless of infection or exercise condition. In the fructose-fed rats both the S. typhimurium and forced exercise increased heart weight significantly; this exercise and disease effect was not noted in the sucrose-fed rats. These fructose effects were even more pronounced when heart-body weight ratios were calculated.

Infection and infection + exercise increased cardiac protein synthesis (RNA, protein, cathepsin D); greatest increase occurred in the sucrose-fed rats. When rats were forced to exercise day 5 post inoculation and sacrificed on day 9 the forced activity appeared to result in greater magnitudes of change than when the animals were sacrificed immediately after exercising.

In one trial in this series the dosage of S. typhimurium administered was double that usually used in our experiments. This resulted in 100% mortality at 6 days p.i. in the groups fed fructose and only 50% in the sucrose-fed rats.

15.5 Forced Exercise x Disease x Fasting

This was the first in a series of experiments to determine if metabolic alterations due to fasting in rats challenged with a mild S. typhimurium infection would also occur during the stress of 2 hours of forced running. Weanling rats were fasted for 24 hours beginning day 6 p.i. and forced to run for 2 hours on day 7 and were then immediately sacrificed.

Plasma glucose was decreased more in fasted controls than in infected rats. Liver glycogen was depleted by both fasting and exercise, with no disease interaction. Hormonal alterations associated with energy metabolism were apparent in the changes in the insulin:glucagon ratio which was decreased 65% by both fasting and exercise and correlated with the metabolic flux of energy mobilization due to the two types of stress; there were no effects of disease on this ratio.

Further results from this series of experiments are included in the accompanying Annual Report and the Ph.D. thesis of Douglas Balentine.

15.6 Interaction of Carbohydrate Source, Linoleic Acid and S. typhimurium

The purpose of these trials was to observe whether the hypertriglyceridemia induced by feeding a 45% fructose diet could be modified by the addition of 10% linoleic acid. Glucose-fed weanling rats were used as controls and all diet groups contained both non-infected and S. typhimurium-infected rats.

Fructose, regardless of linoleic acid level, was as efficient a

source of energy as glucose in non-infected animals; this was not the case in the infected rats. Fructose feeding with low linoleic acid produced hypertriglyceridemia, an effect which was overcome when 10% linoleic acid was added to the diet. Fructose-fed animals had larger livers and spleens than those fed glucose, but this effect was masked in the S. typhimurium-infected rats because the infection also produced larger livers and spleens. The data appear in full in the Ph.D. thesis of Hallis Kenler.

15.7 Effect of 5/6 Nephrectomy on Voluntary Wheel Running in Rats

Weanling rats were given constant voluntary access to running wheels, fed a balanced diet ad libitum or 65% of ad libitum (to induce wheel running) and subjected to a 5/6 nephrectomy, sham operation or left intact. At the end of 12 days all rats were sacrificed. Control and sham-operated rats on the restricted feeding regimen had identical activity curves, with maximum running on day 5. The activity curve for the nephrectomized rats was identical to the others but wheel turns averaged 60% below the control and sham-operated groups. The nephrectomy did not induce wheel running in the ad libitum-fed rats, as had been hypothesized.

15.8 Potential Significance of Carnosine in the Infected Tissues of Rats and Chicks

Concentrations of anserine, carnosine and free histidine were determined in muscle tissue of male chicks inoculated with low and high levels of avian tuberculosis and in weanling rats infected with S. typhimurium. Both infections resulted in a decrease in carnosine concentrations. On the other hand, anserine concentrations were not affected by either the low or high levels of infection. Tissue free histidine increased significantly due to high levels of infection, with no apparent response to low infection levels. The results are consistent with the hypothesis that carnosine acts as a tissue reservoir for histidine, ultimately serving as a precursor of histamine.

15.9 Lysine Deficiency and Voluntary Exercise in Chicks

Young chicks were given constant voluntary access to running wheels and fed a balanced diet or one deficient in lysine. Whenever body weights were significantly ($P < 0.01$) different between the two groups the diets were switched. When the lysine-deficient diet was offered the chicks increased their running activity. This was in line with earlier observations made on the rat where a lysine-deficient diet also increased running activity. The data clearly demonstrated how a nutritional imbalance can result in a change in activity behavior. The data appear in the Master's thesis of Merrily Licwinko.

15.10 Carbohydrate Source and Forced Exercise in Chicks

In three replicated trials, chicks were fed diets containing either 50% fructose, sucrose or glucose and subjected to forced exercise (running wheels) for 30 min the first day, 45 the second day, and 60 min the third and final day and were then sacrificed immediately. Body weights, liver weights and liver/body weight ratios were lowest in the fructose-fed chicks. Forced exercise depressed body weights and reduced total liver fructose, glucose and glycogen but did not cause any significant changes in total liver lipid fractions.

The data emphasized once again that dietary energy source should be considered in studies involving exercise or disease.

15.11 Uptake and Metabolism of Fructose during Dietary Loading of Chicks Infected with Avian Tuberculosis

The results of this research indicated that fructose required a period of dietary adaptation before it was fully utilized by the chick. This period was marked by poor feathering, watery feces and depleted energy stores and high levels of plasma fructose. Since there were no changes in levels of fructose-1-phosphatase in the liver, fructokinase and aldolase apparently were not involved. Both control and tuberculosis-infected chicks fed 45% fructose had greater depletion of energy stores than those fed glucose. This lower energy level correlated with a low degree of TB involvement. An interaction was observed between sugar used and TB with respect to liver triglyceride and glycogen levels. The data appear in full in the Ph.D. thesis of Minu Chaudhuri.

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Charles Taylor, Laboratory Technician, Full time
Catherine DiGioia " " "

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